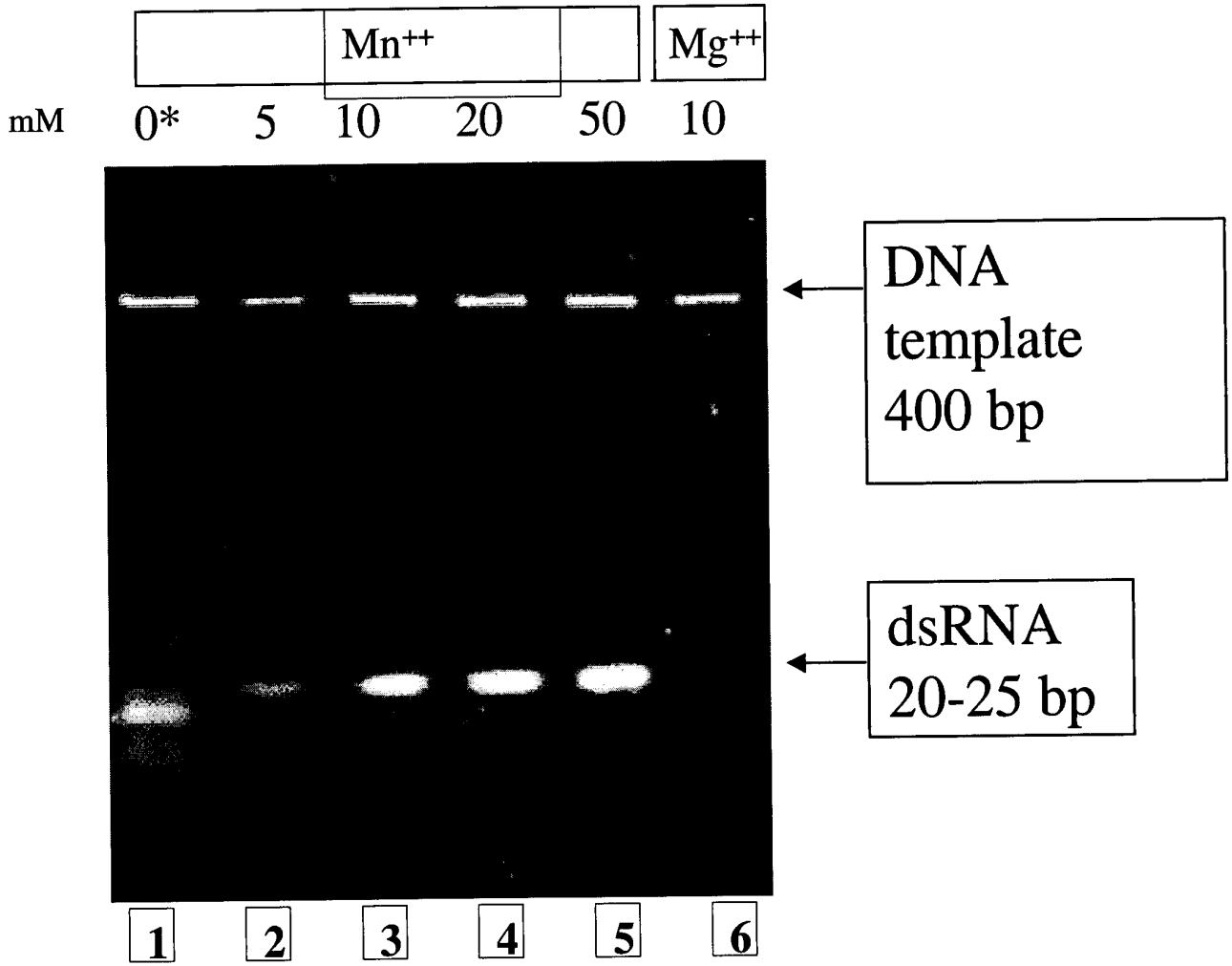


Fig.1A

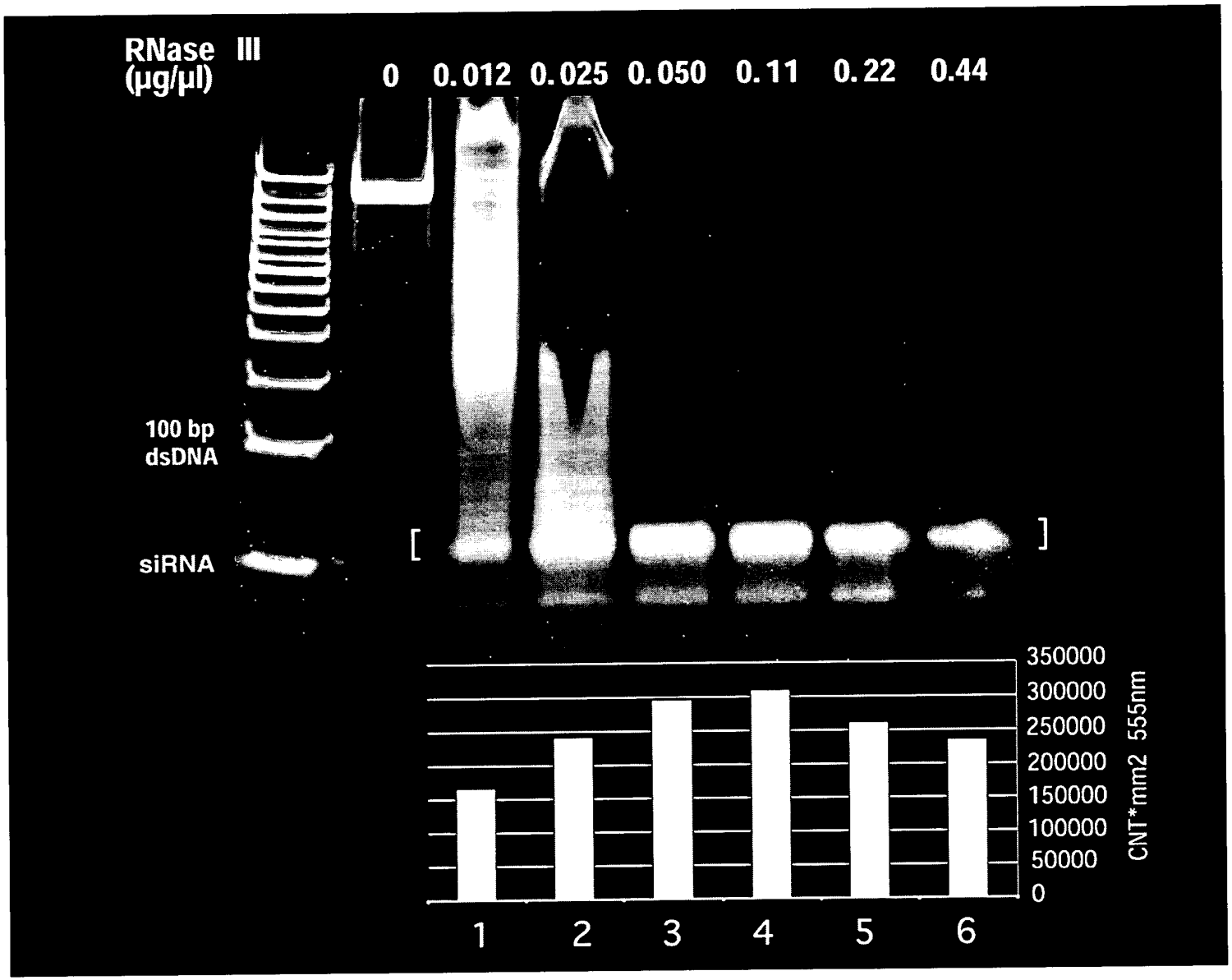


Fig.1B

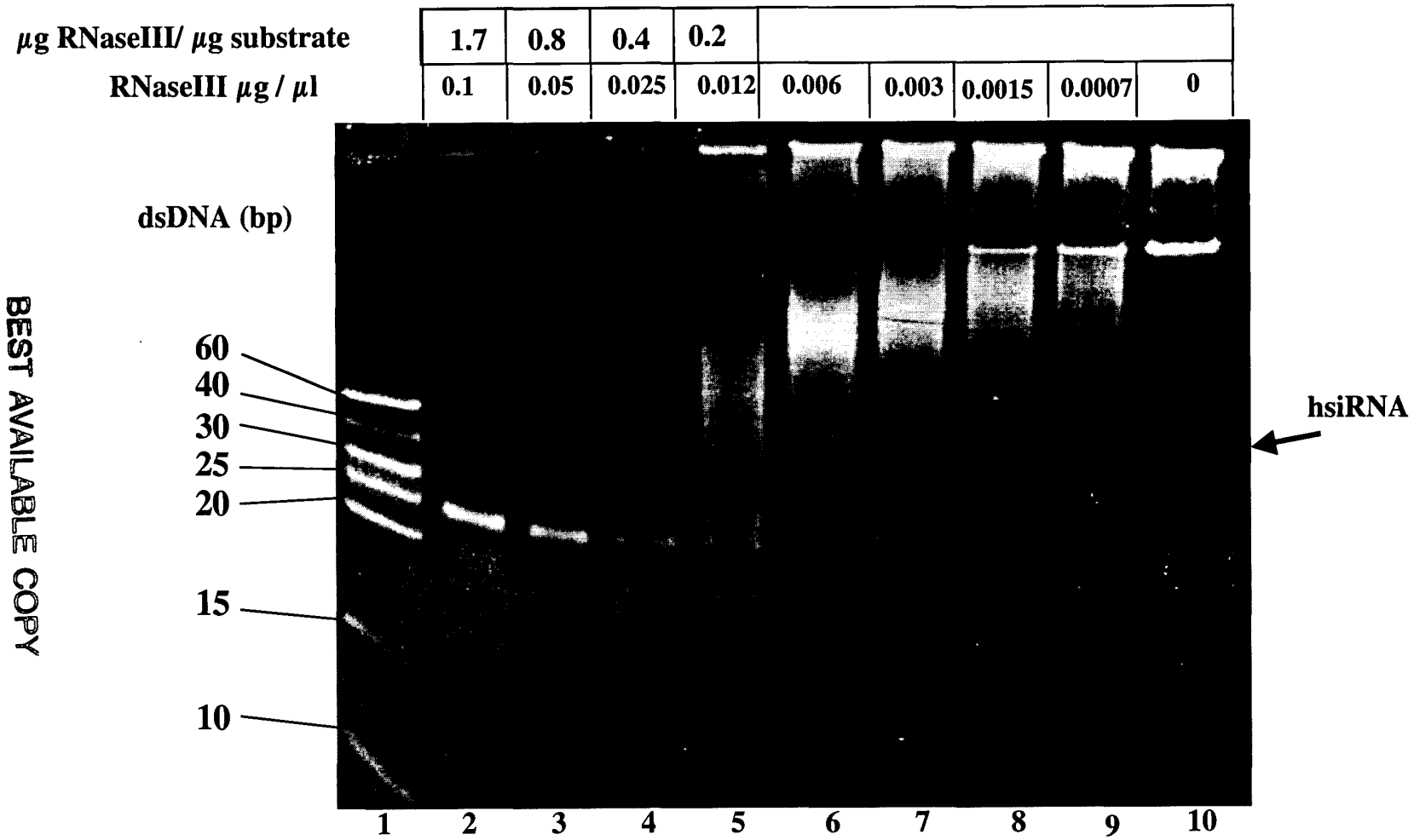


Fig.1C

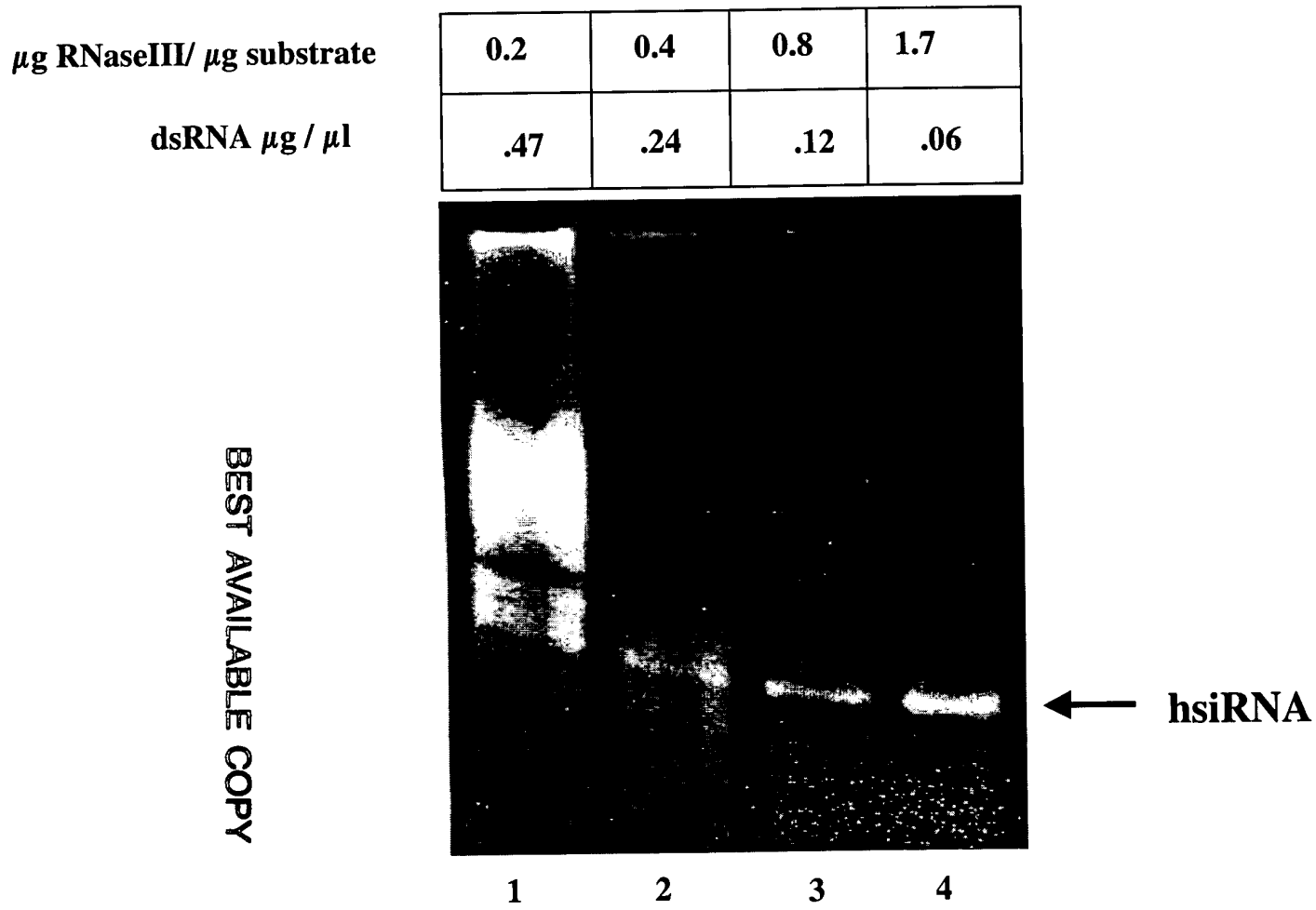


Fig.1D

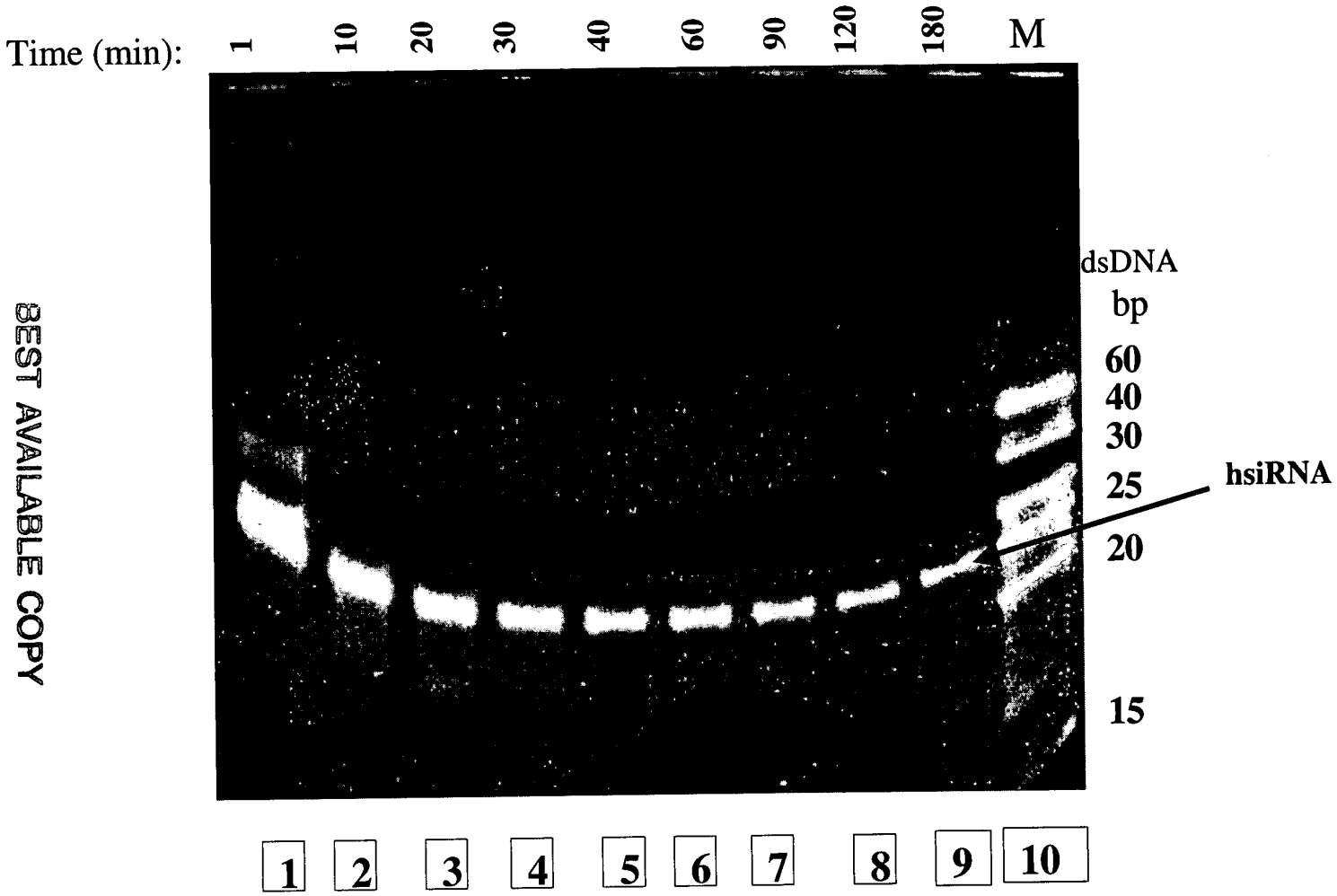


Fig.1E

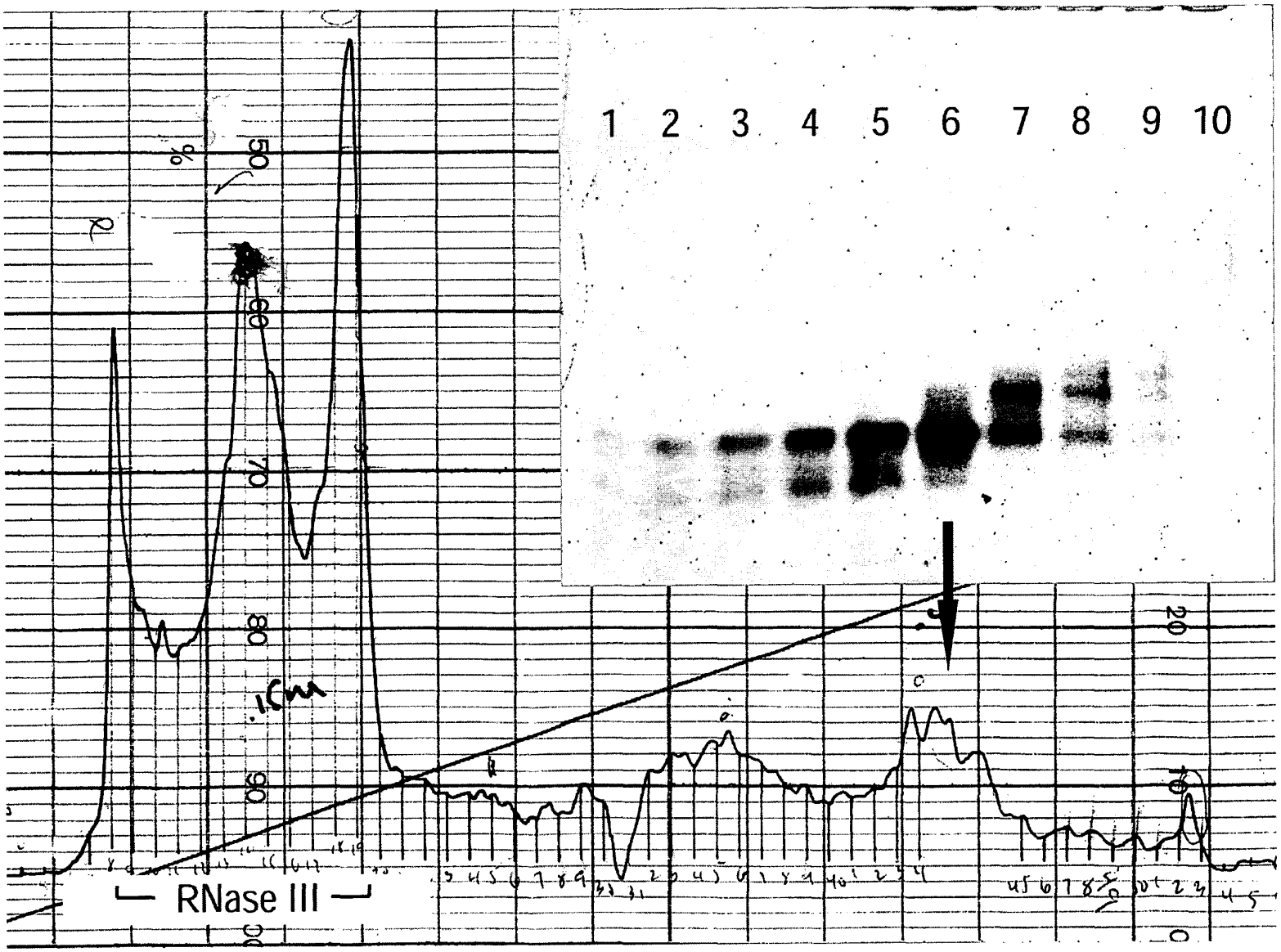


Fig. 1F

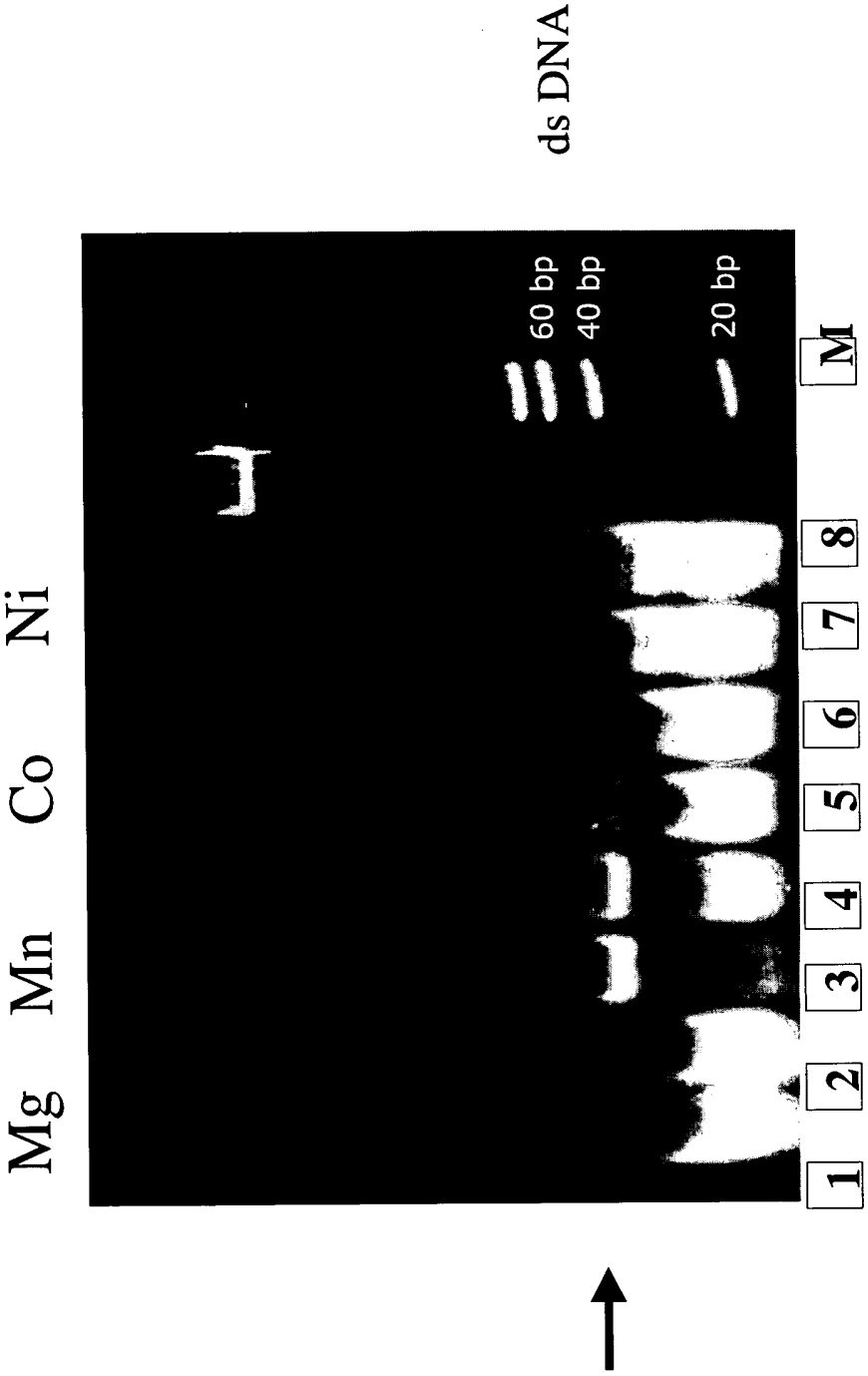


Fig. 2

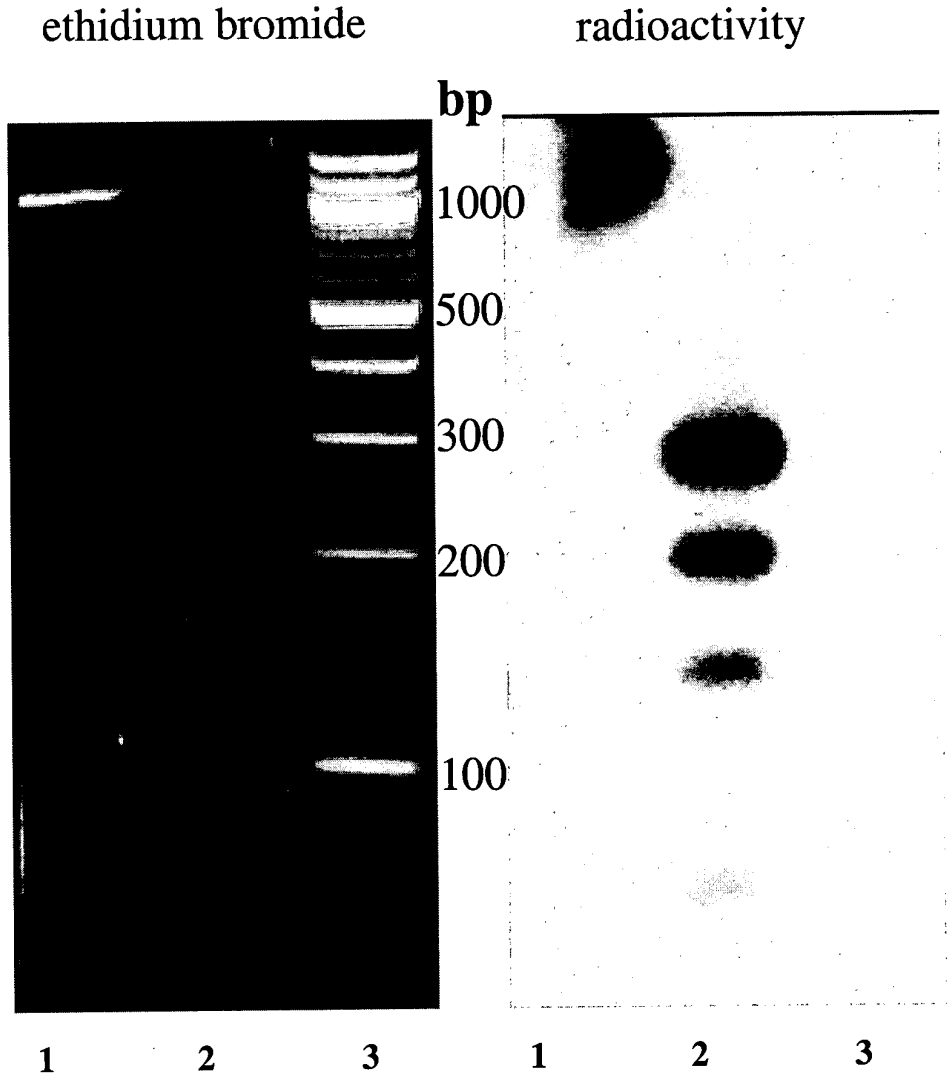


Fig. 3A

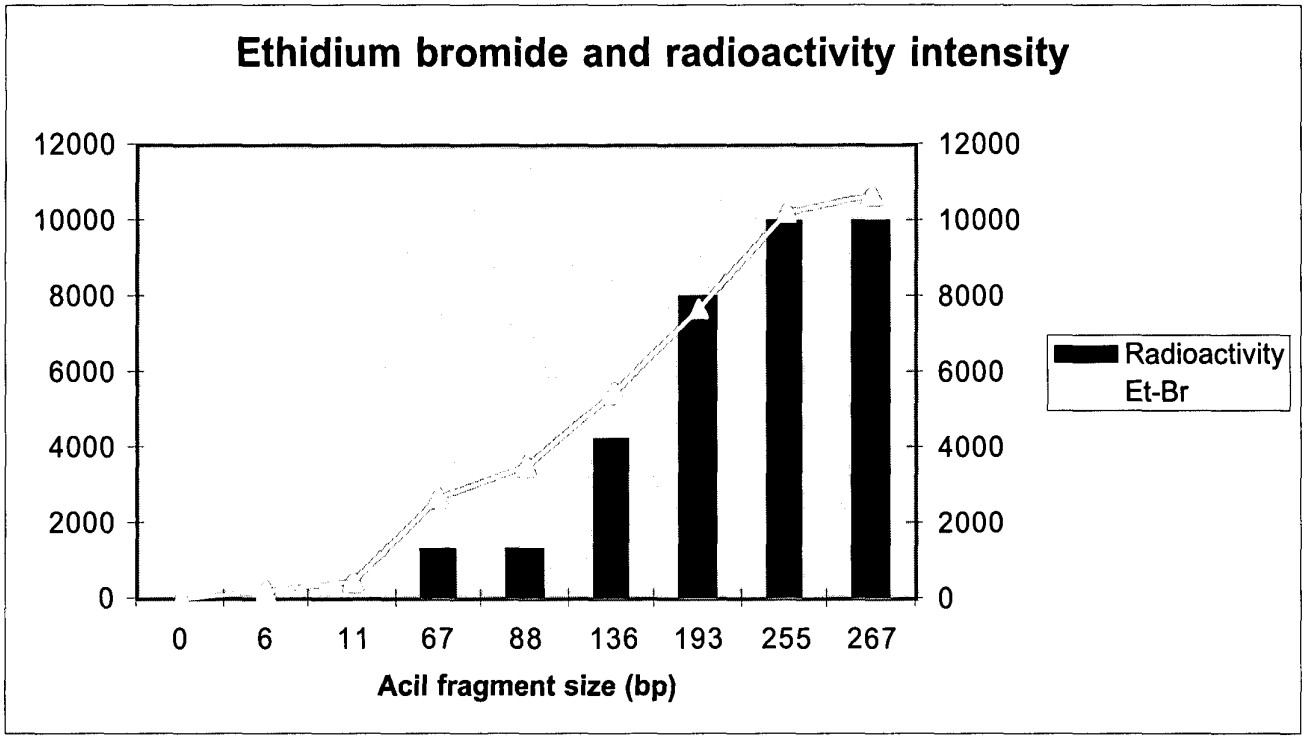

Ethidium bromide and radioactivity intensity
12000
10000
8000
6000
4000
2000
0
0
6
11
67
88
136
193
255
267
Acil fragment size (bp)
Radioactivity
Et-Br

Fig. 3B

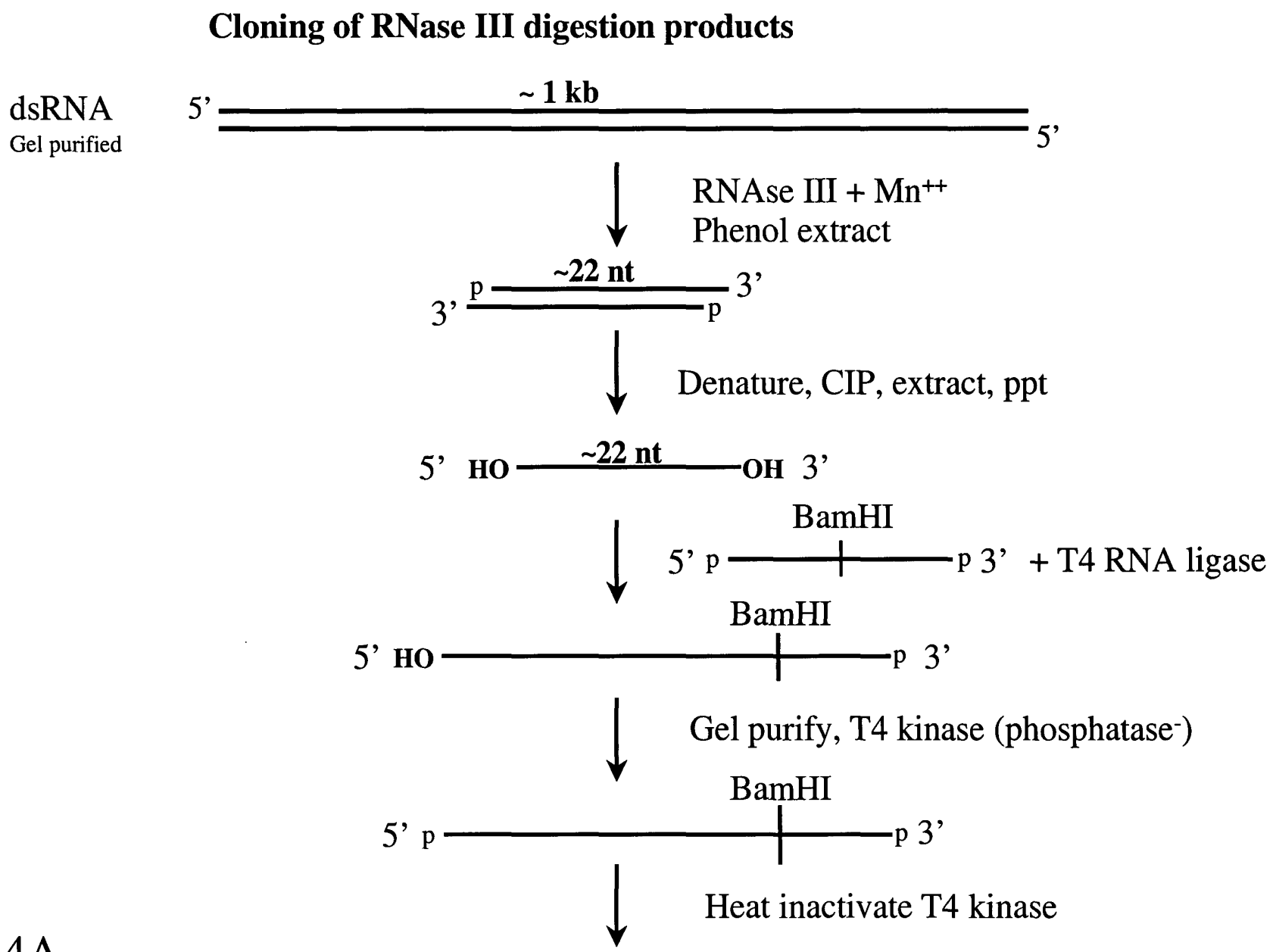

Cloning of RNase III digestion products
dsRNA
Gel purified
5'
~ 1 kb
5'
RNAse III + Mn++
Phenol extract
~22 nt
p
3'
3'
p
Denature, CIP, extract, ppt
5' HO
~22 nt
OH 3'
BamHI
5' p
p 3' + T4 RNA ligase
BamHI
5' HO
p 3'
Gel purify, T4 kinase (phosphatase-)
BamHI
5' p
p 3'
Heat inactivate T4 kinase

Fig. 4A

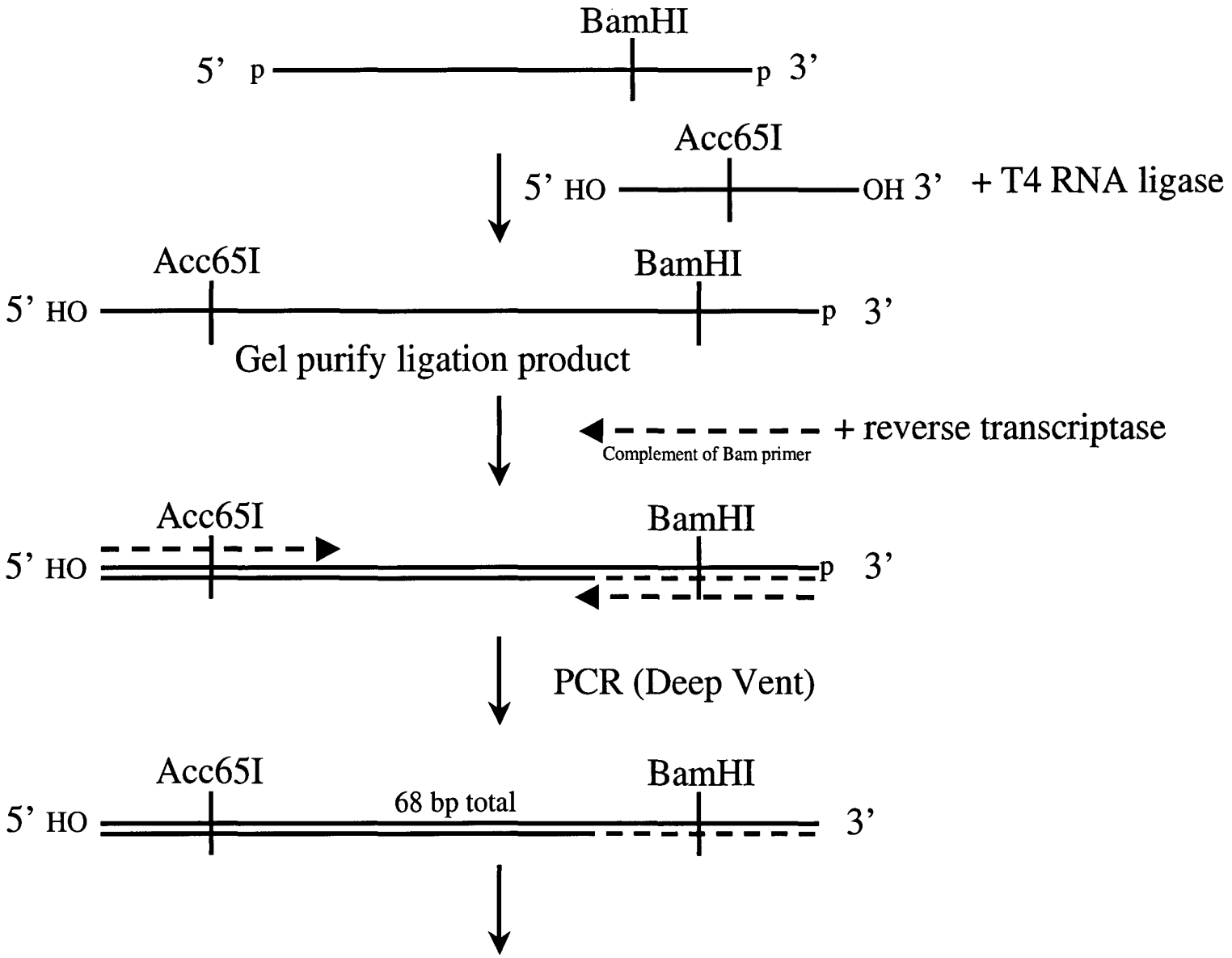

BamHI
5' p
p 3'
Acc65I
5' HO
OH 3'
+ T4 RNA ligase
Acc65I
BamHI
5' HO
p 3'
Gel purify ligation product
+ reverse transcriptase
Complement of Bam primer
Acc65I
BamHI
5' HO
p 3'
PCR (Deep Vent)
Acc65I
BamHI
68 bp total
5' HO
3'
Digest library with Acc65I and BamHI, clone into pUC19

Fig. 4A (cont.)

Mapping cloned RNA fragments onto original malE transcript

BglII-EcoRI malE fragment cloned in LITMUS 28i:

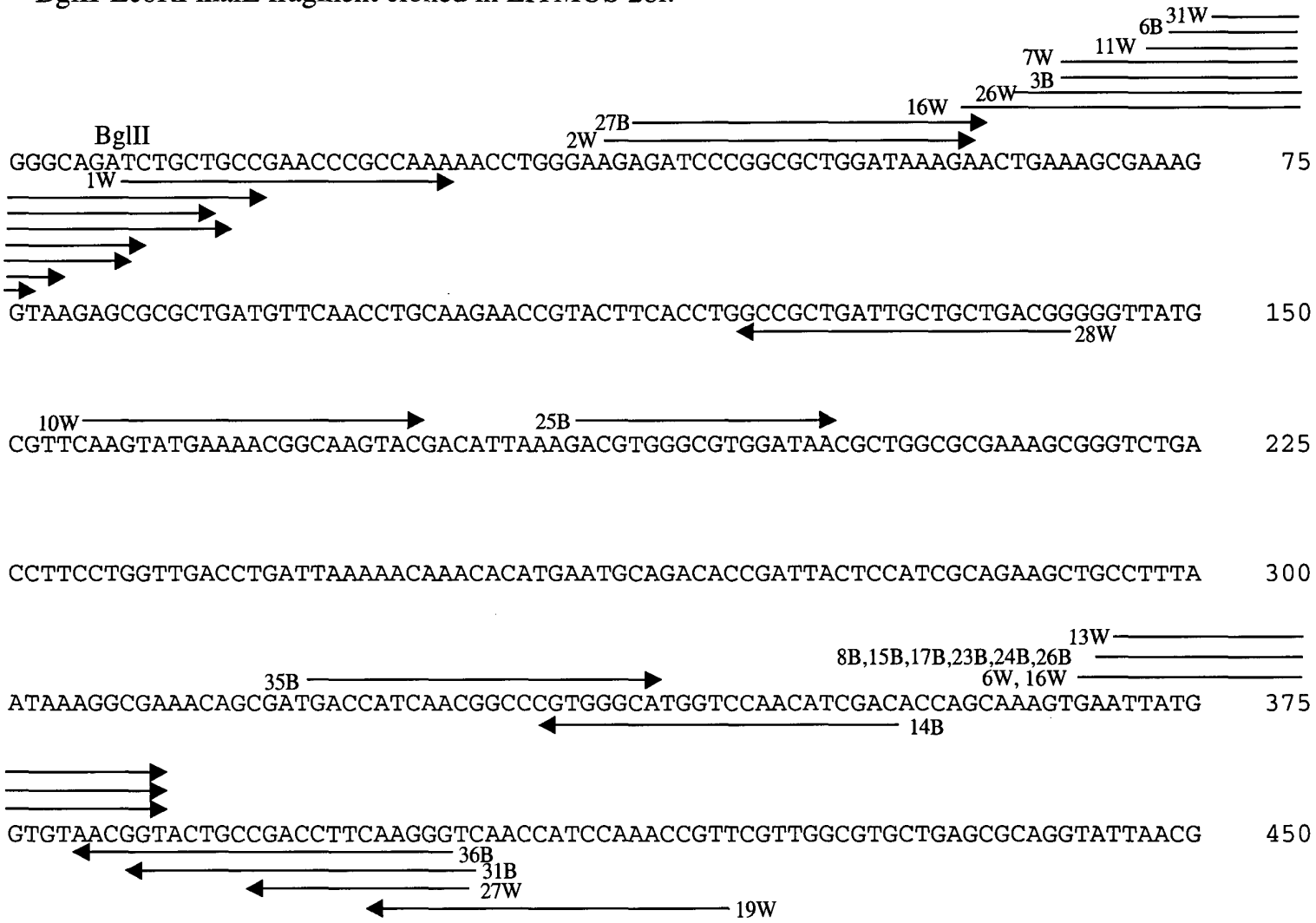


Fig. 4B

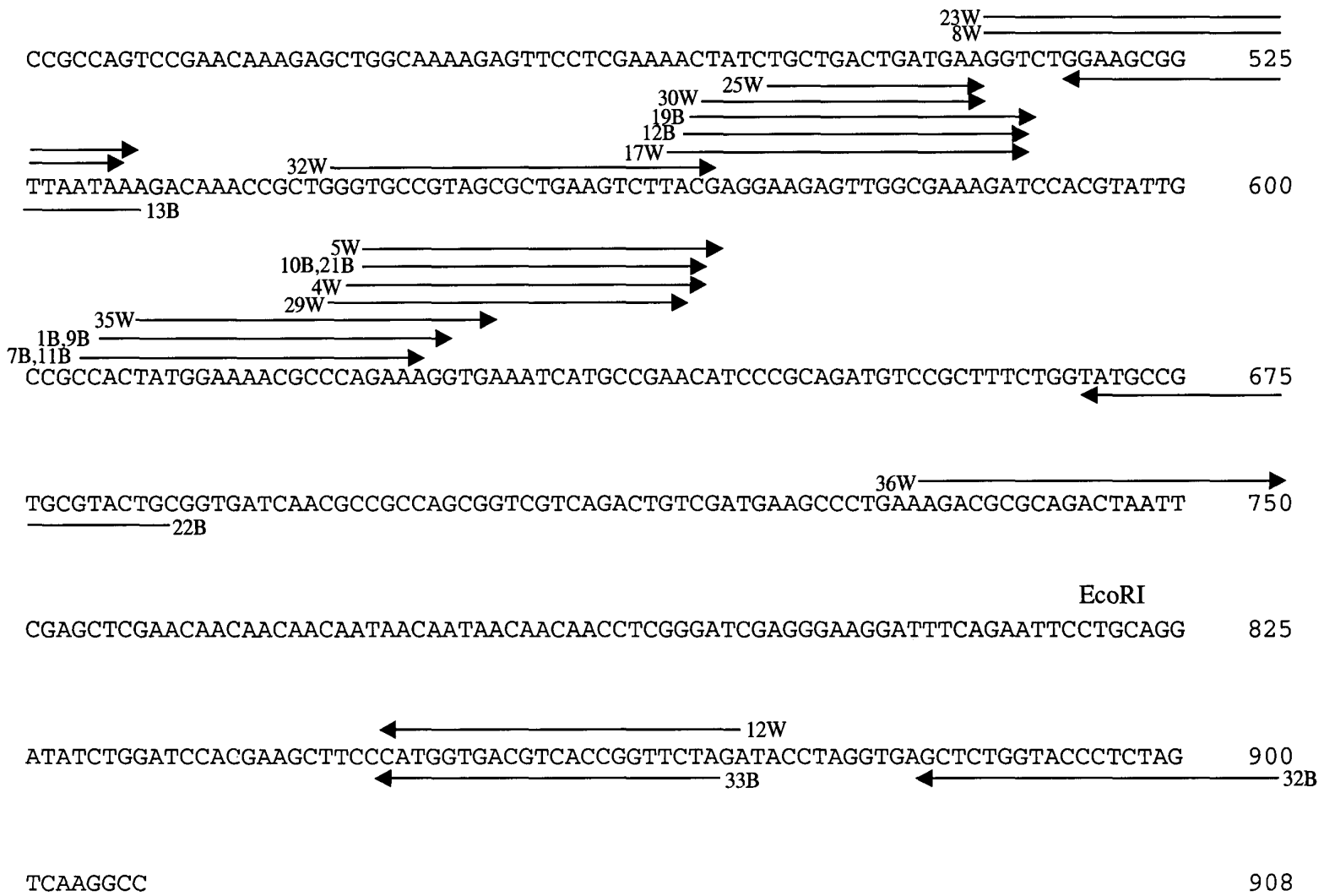

23W
8W
CCGCCAGTCCGAACAAAGAGCTGGCAAAAGAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGGAAGCGG 525
25W
30W
19B
12B
17W
32W
TTAATAAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTG 600
13B
5W
10B,21B
4W
29W
35W
1B,9B
7B,11B
CCGCCACTATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCCGCTTTCTGGTATGCCG 675
36W
TGCGTACTGCGGTGATCAACGCCGCCAGCGGTCGTCAGACTGTCGATGAAGCCCTGAAAGACGCGCAGACTAATT 750
22B
EcoRI
CGAGCTCGAACAACAACAACAATAACAATAACAACAACCTCGGGATCGAGGGAAGGATTTCAGAATTCCTGCAGG 825
12W
ATATCTGGATCCACGAAGCTTCCCATGGTGACGTCACCGGTTCTAGATACCTAGGTGAGCTCTGGTACCCTCTAG 900
33B
32B
TCAAGGCC 908

Fig. 4B (cont.)

Mapping cloned RNA fragments onto original GFP transcript

NheI-BsrGI GFP fragment cloned in LITMUS 38i:

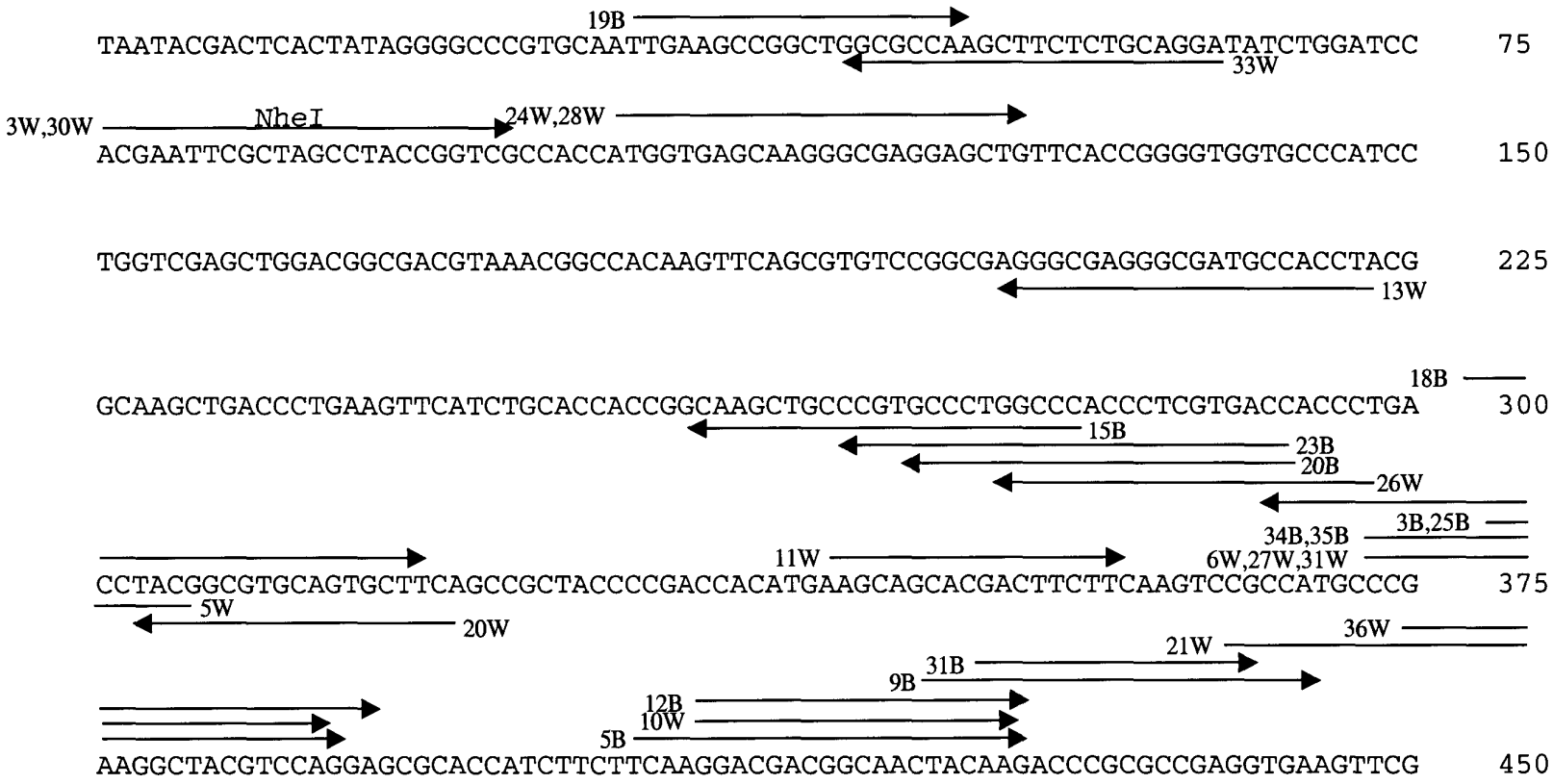


Fig. 4C

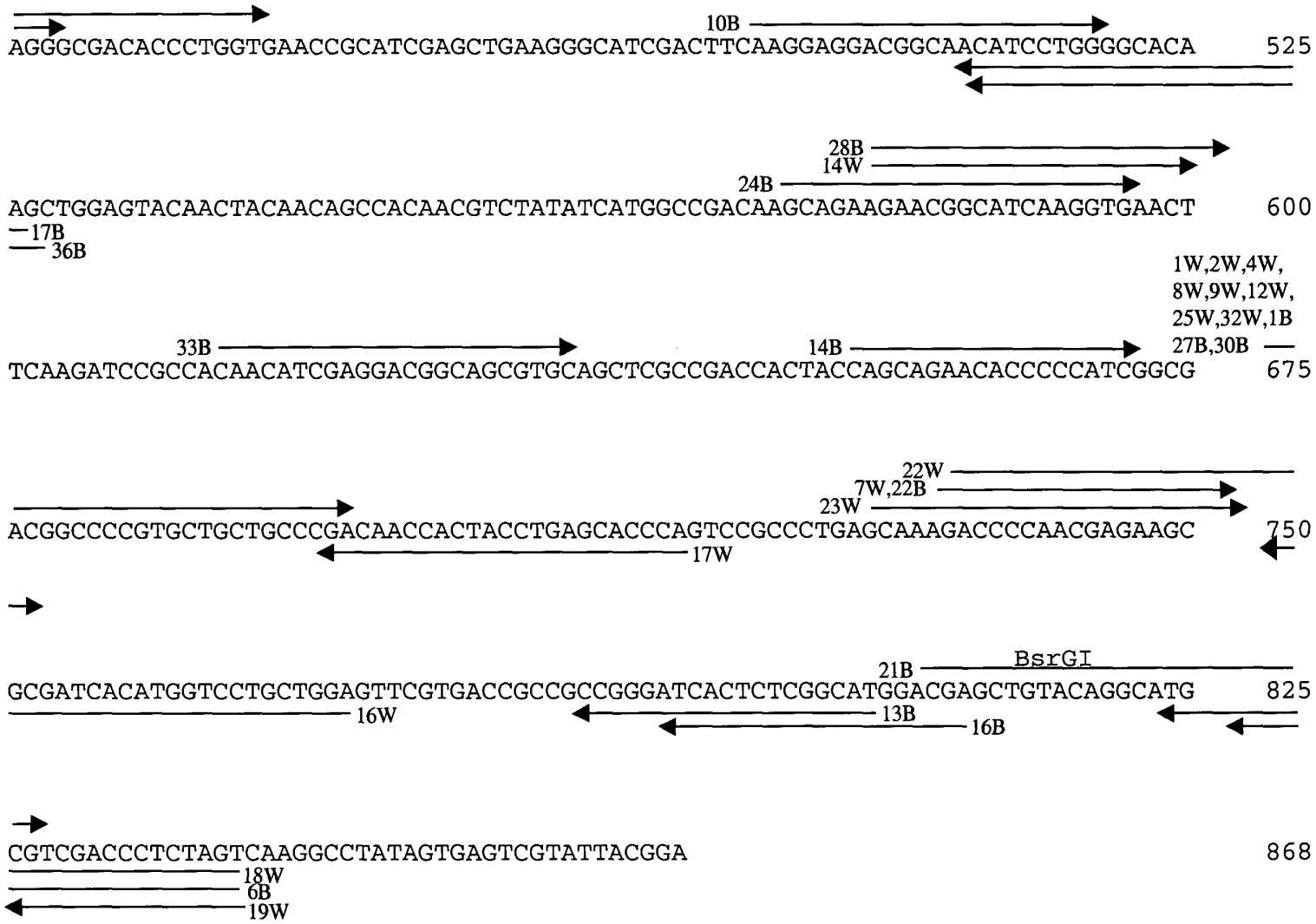
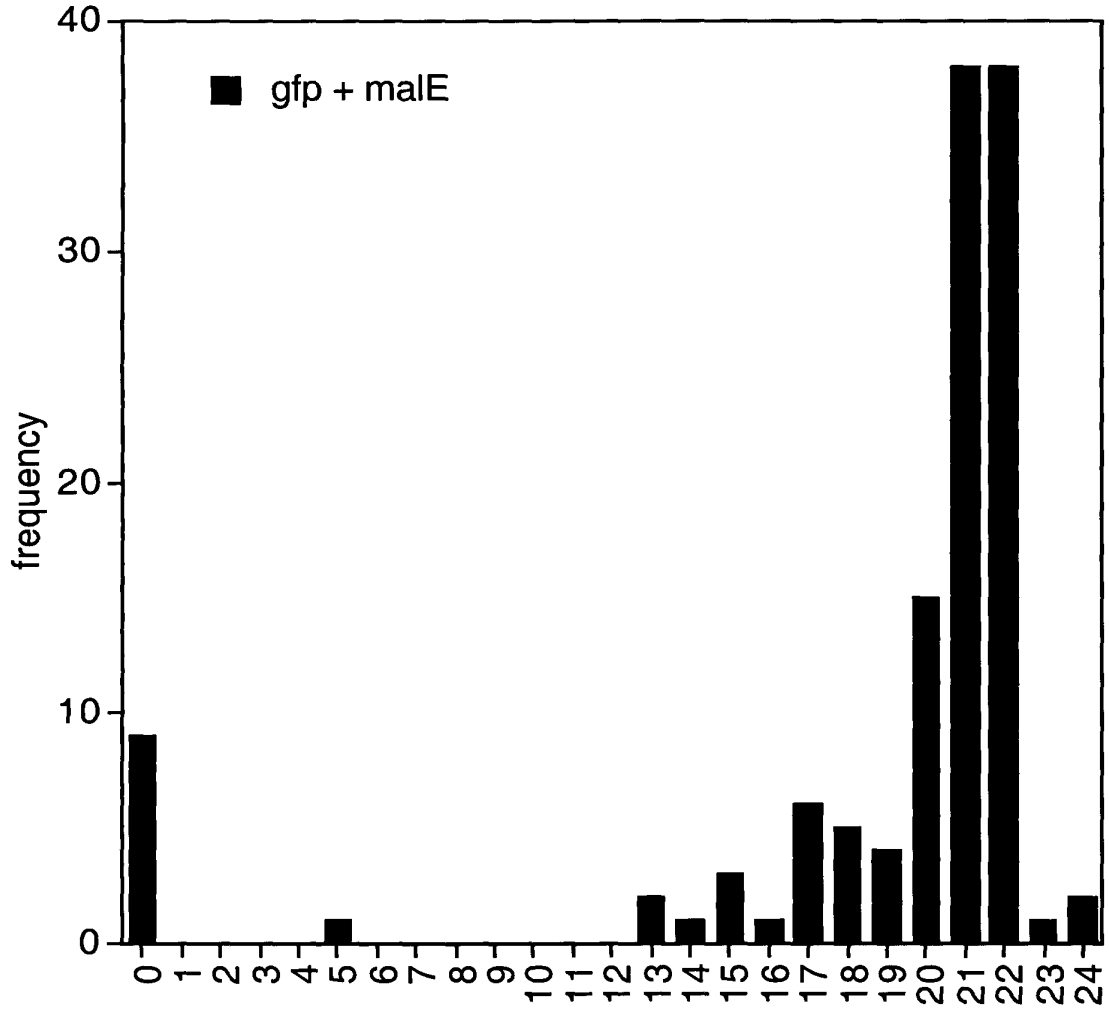

10B
AGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACA 525
28B
14W
24B
AGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT 600
17B
36B
1W,2W,4W,
8W,9W,12W,
25W,32W,1B
27B,30B
33B
14B
TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCG 675
22W
7W,22B
23W
ACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGC 750
17W
BsrGI
21B
GCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAGGCATG 825
16W
13B
16B
CGTCGACCCTCTAGTCAAGGCCTATAGTGAGTCGTATTACGGA 868
18W
6B
19W

Fig. 4C (cont.)

Insert length summary- total clones from both genes



length	frequency
0	9
5	1
13	2
14	1
15	3
16	1
17	6
18	5
19	4
20	15
21	38
22	38
23	1
24	2
total	126

Fig. 4D

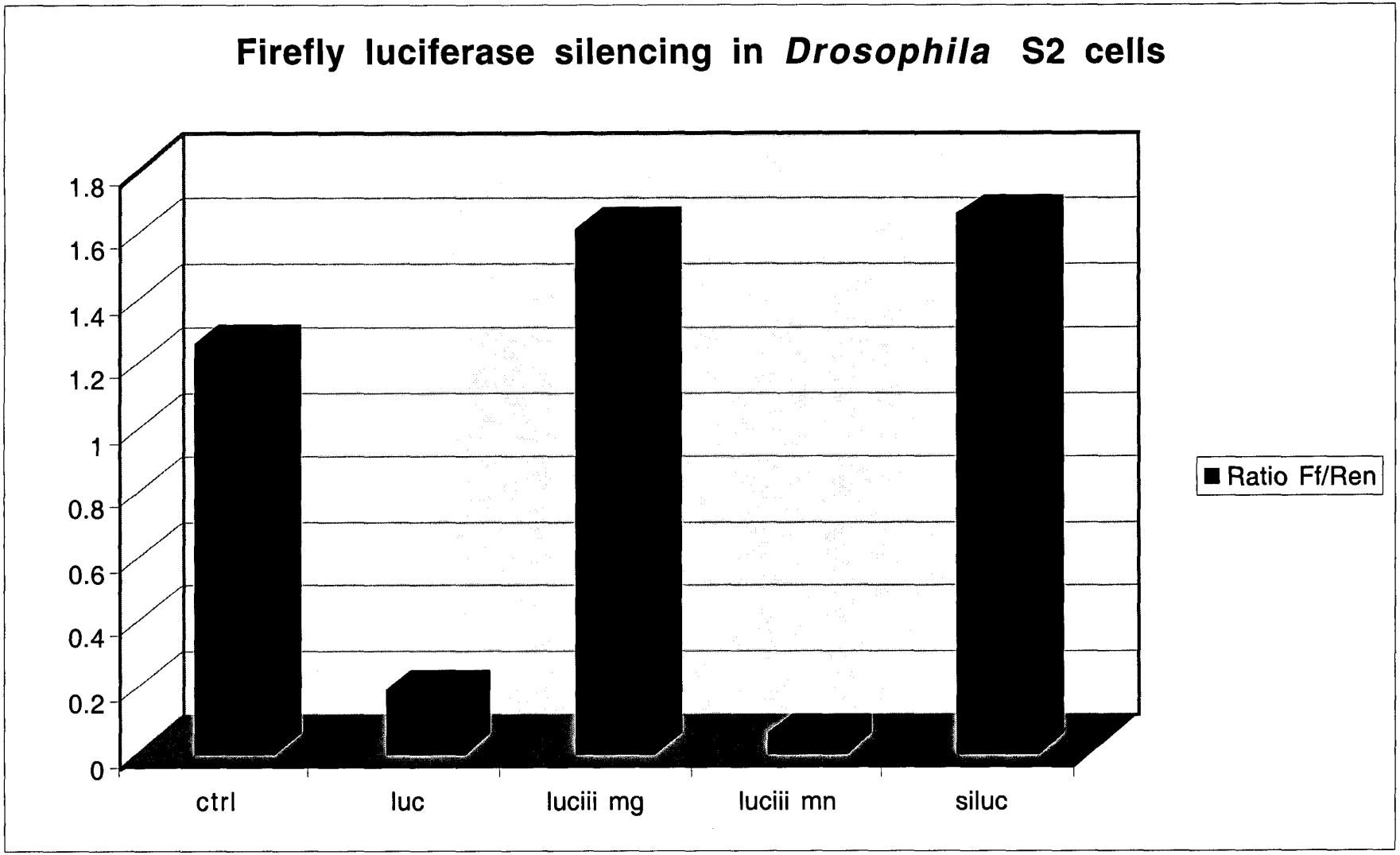

Firefly luciferase silencing in *Drosophila* S2 cells
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
luc
luciii mg
luciii mn
siluc
Ratio Ff/Ren

Fig. 5

EGFP silencing in HEK-293 cells

i

control

ii

GFP dsRNA

RNaseIII Mn++

Fig. 6A

Luciferase is unaffected by GFP hsiRNA in HEK-293 cells

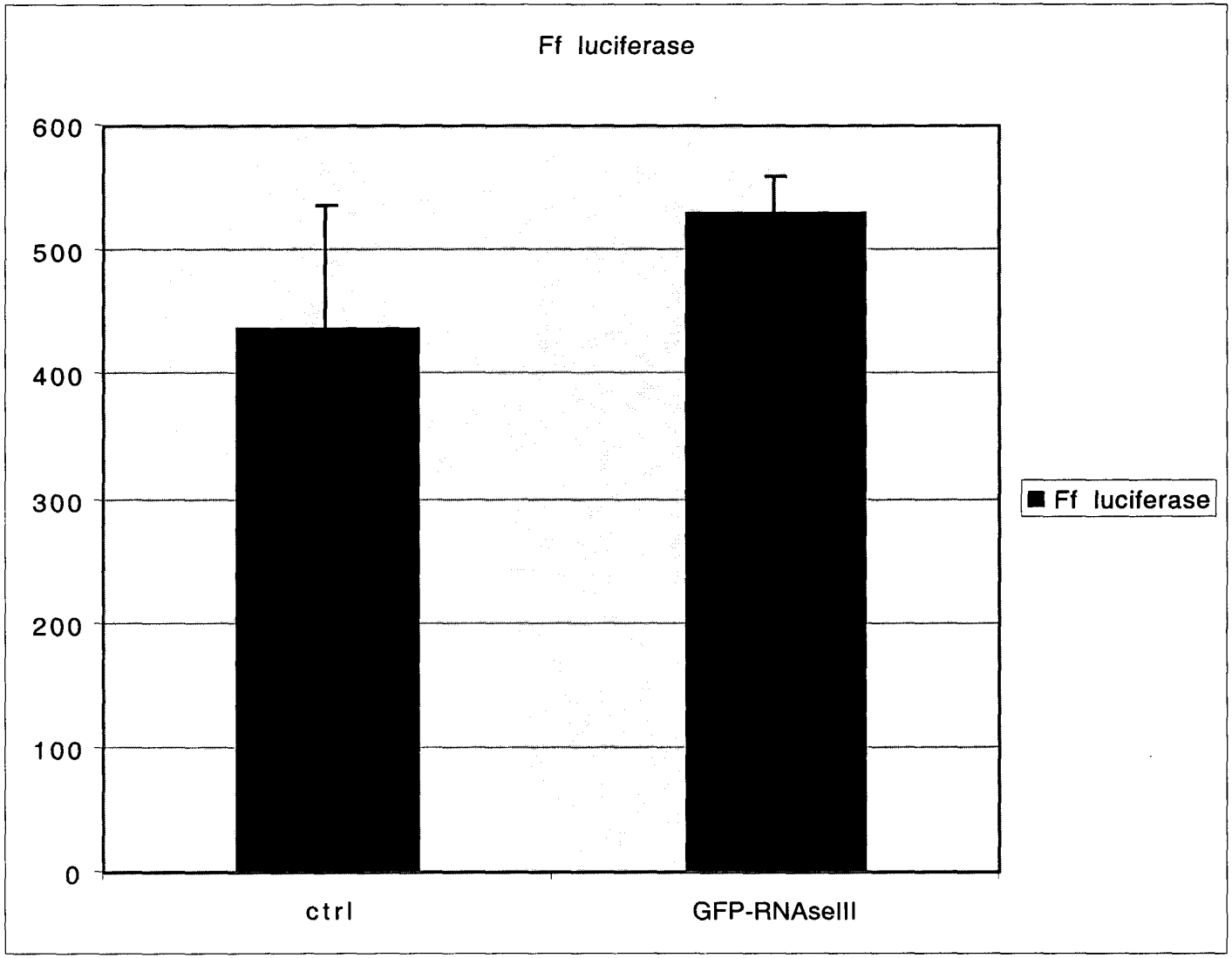


Fig. 6B

Luciferase silencing by luciferase hsiRNA in HEK-293 cells

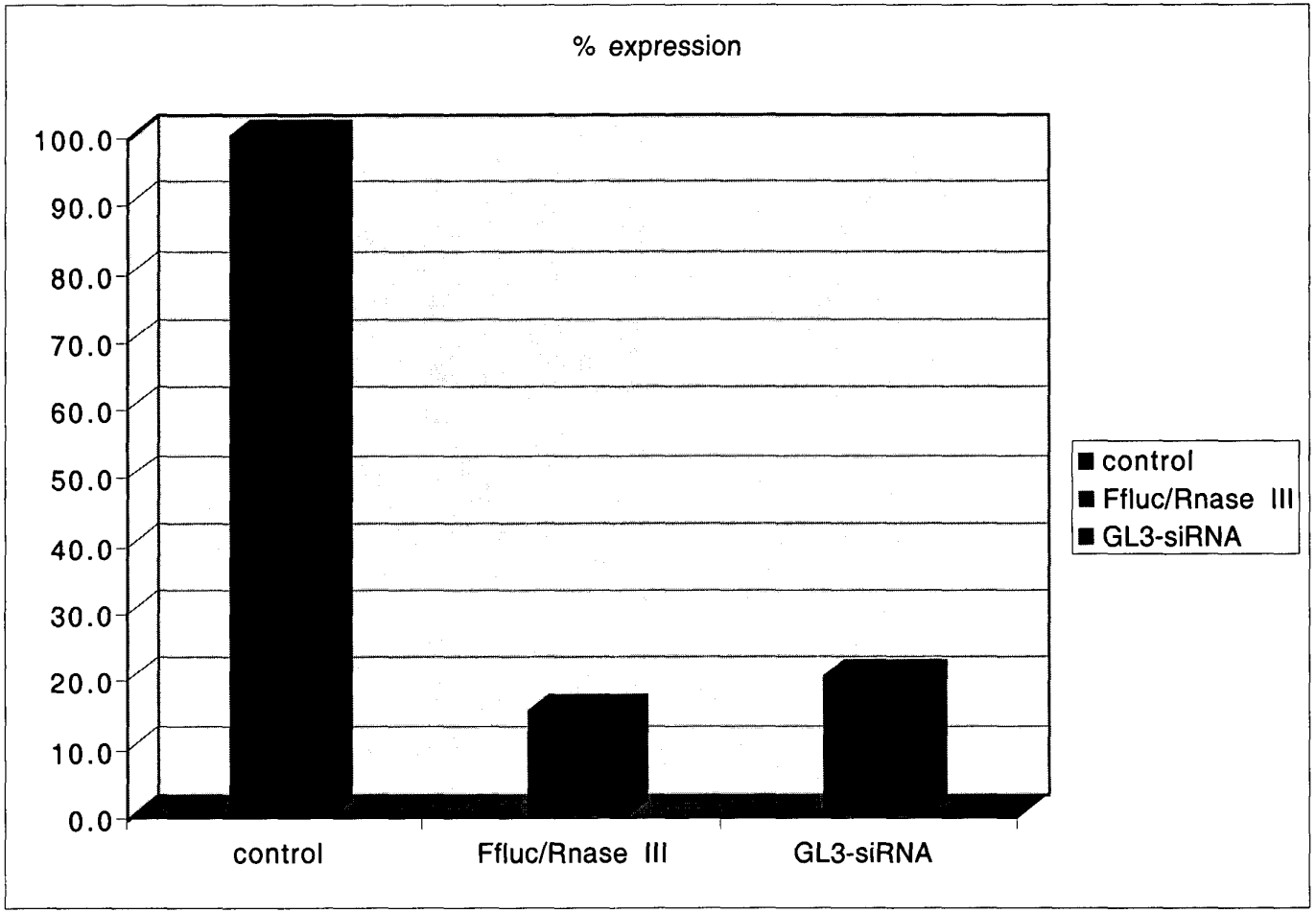


Fig. 6C

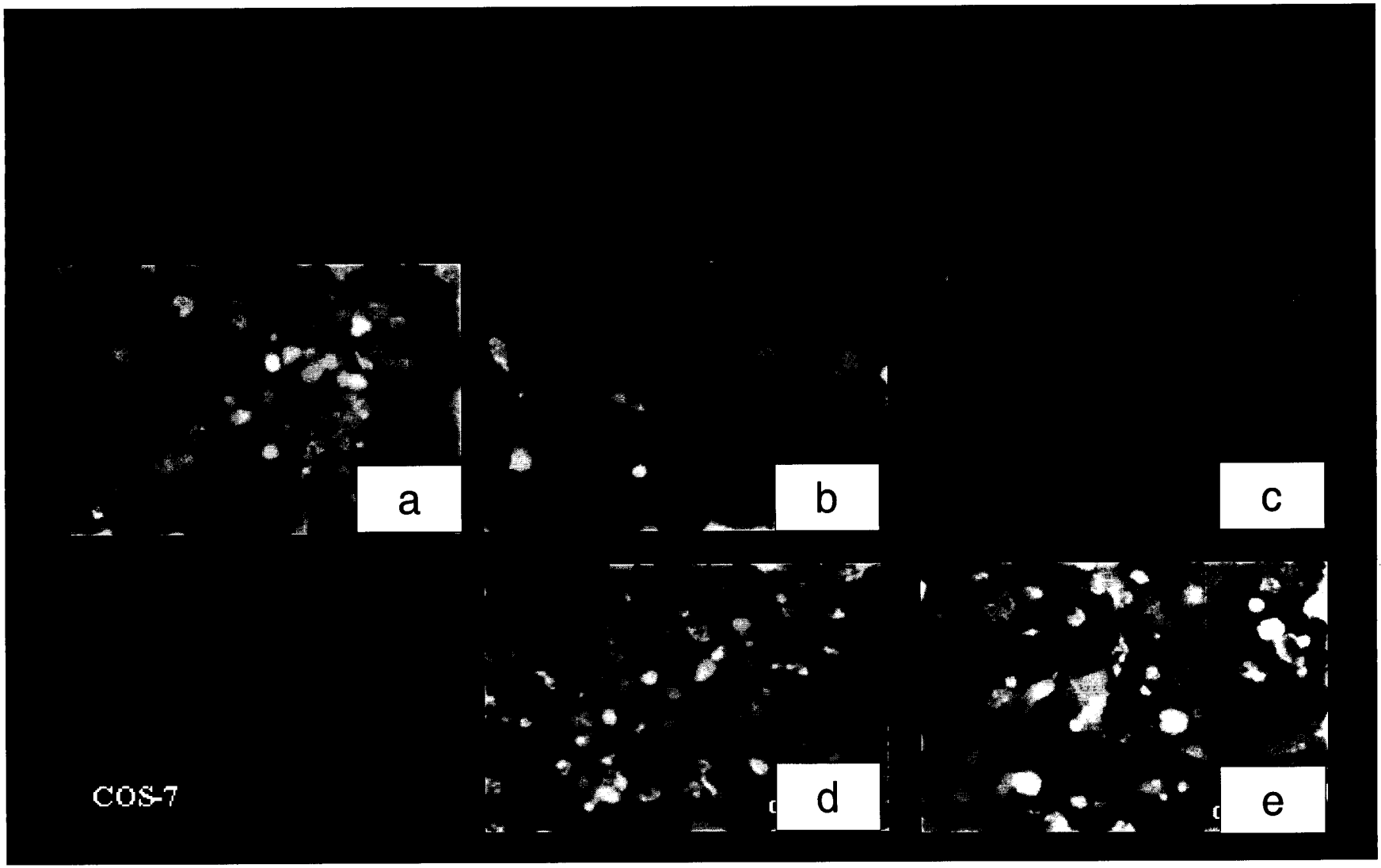


Fig. 7

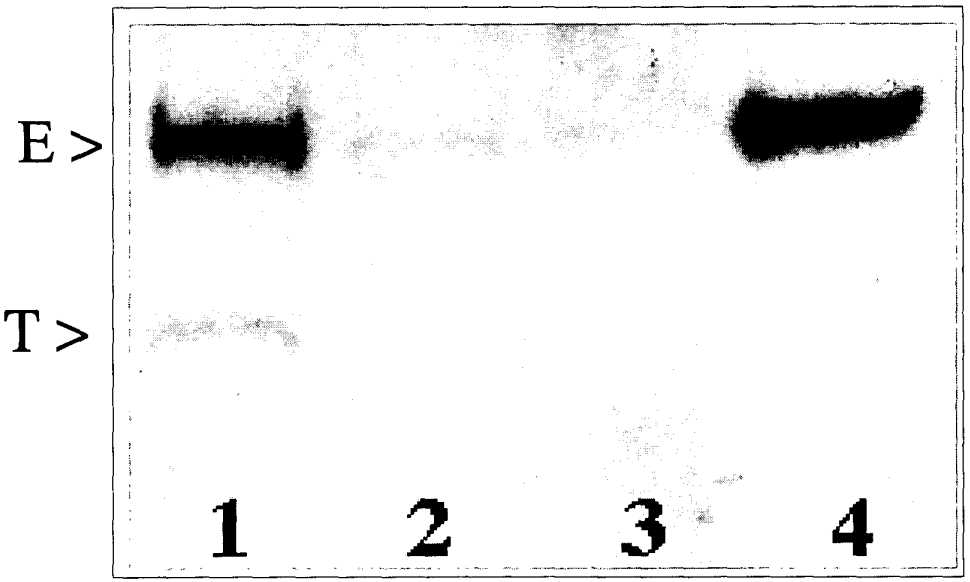


Fig. 8A

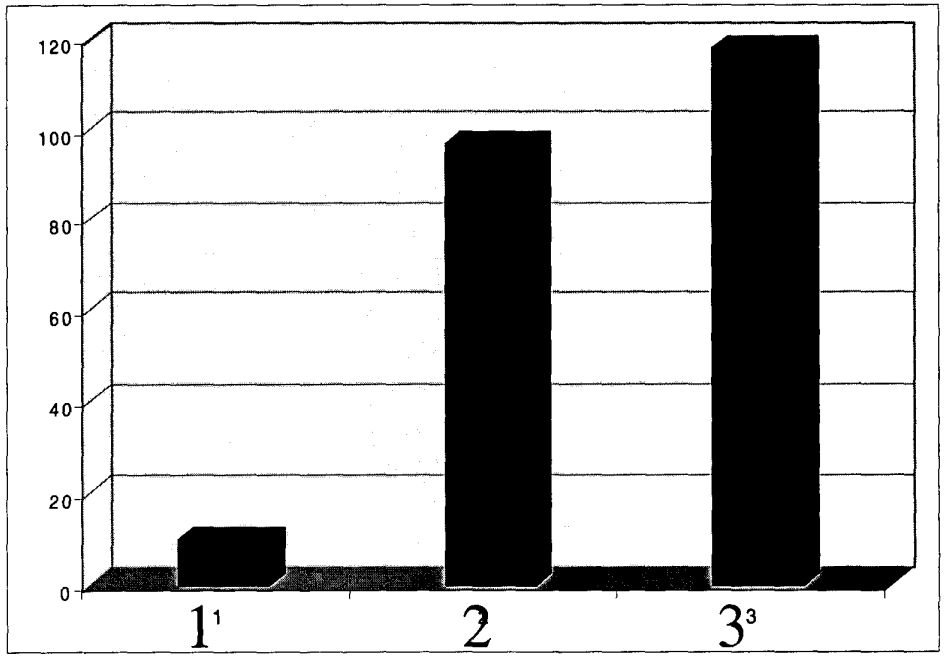


Fig. 8B

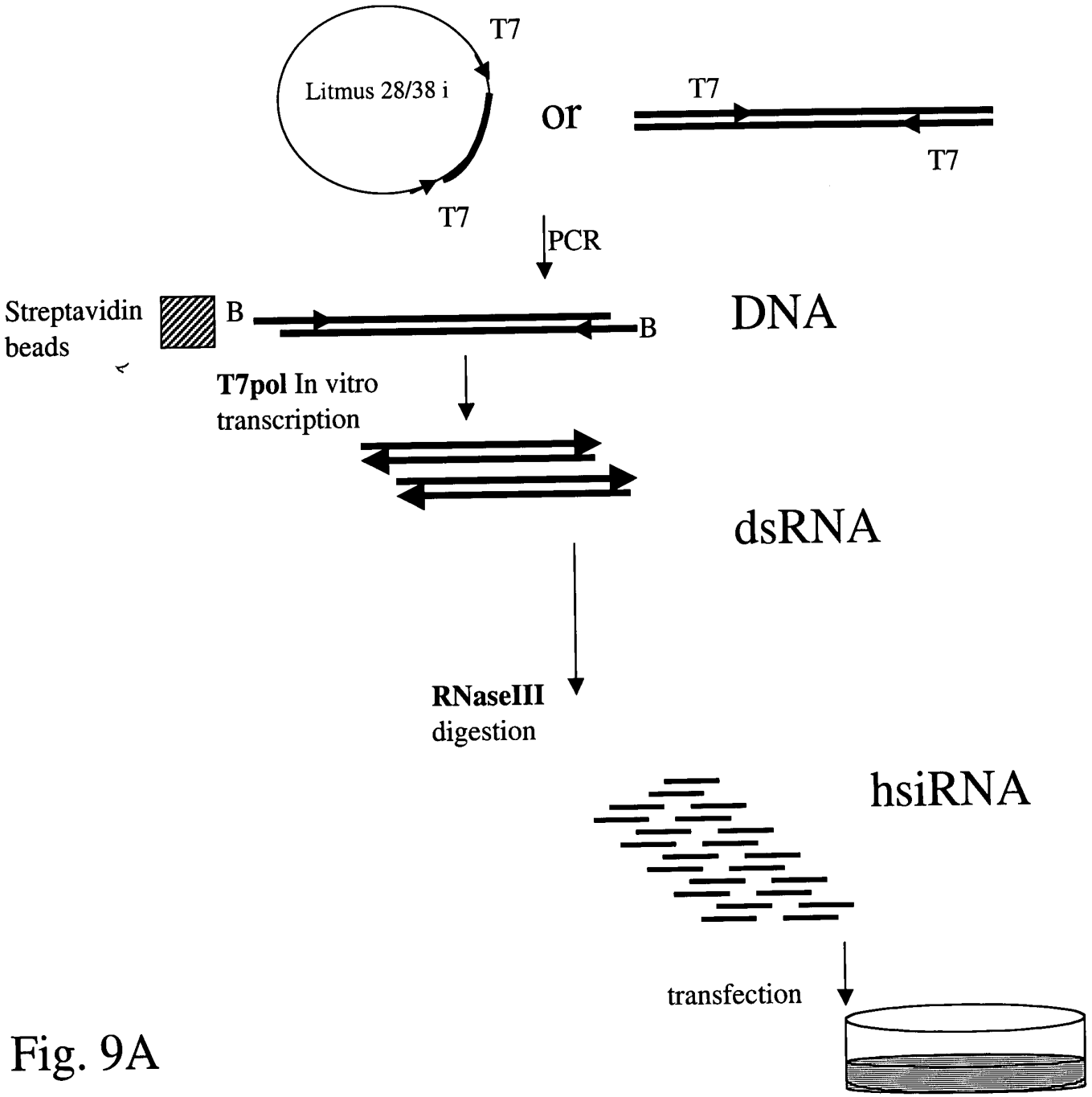

T7
Litmus 28/38 i
T7
or
T7
T7
PCR
Streptavidin beads
B
B
DNA
T7pol In vitro transcription
dsRNA
RNaseIII digestion
hsiRNA
transfection

Fig. 9A

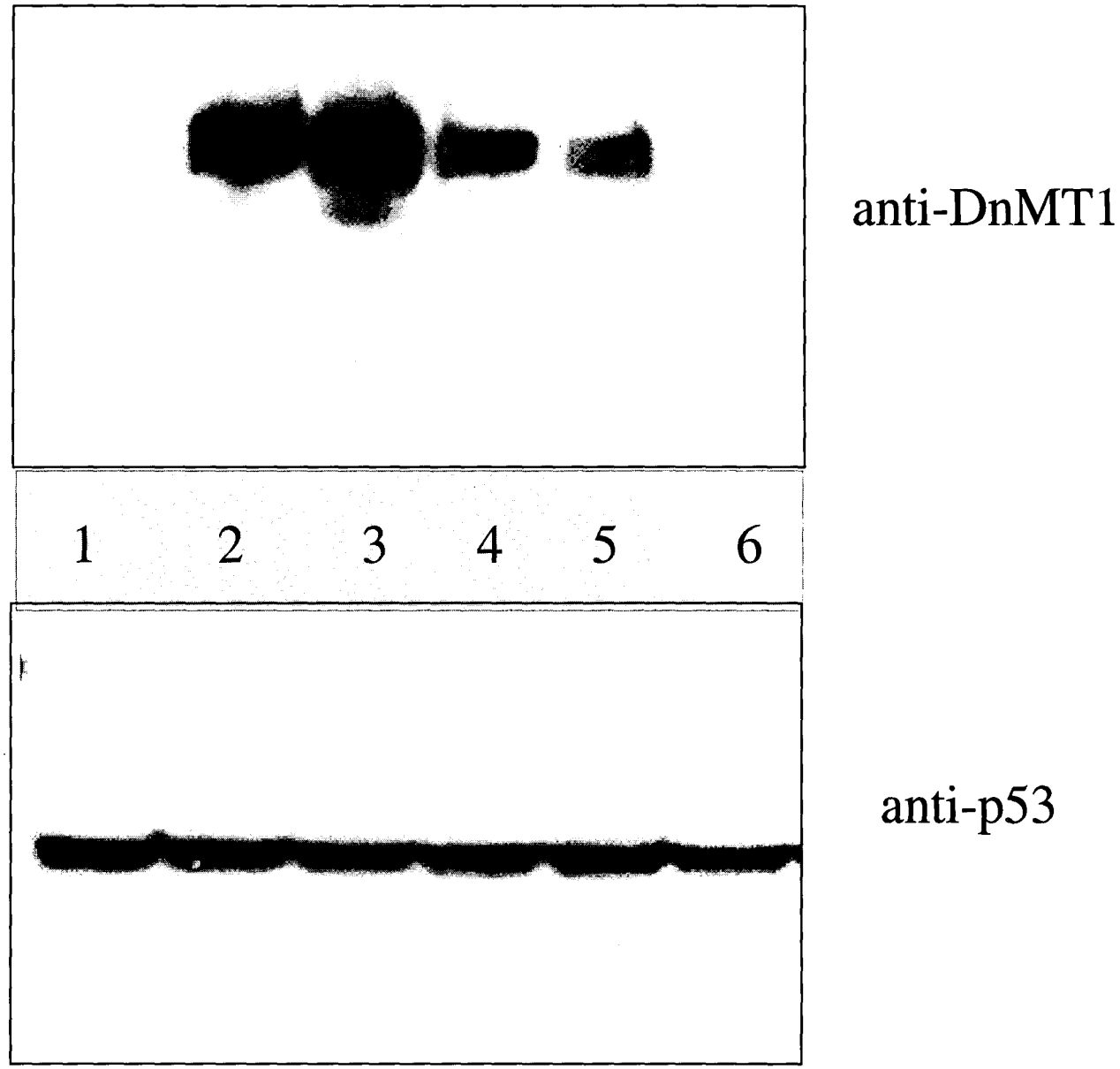


Fig. 10

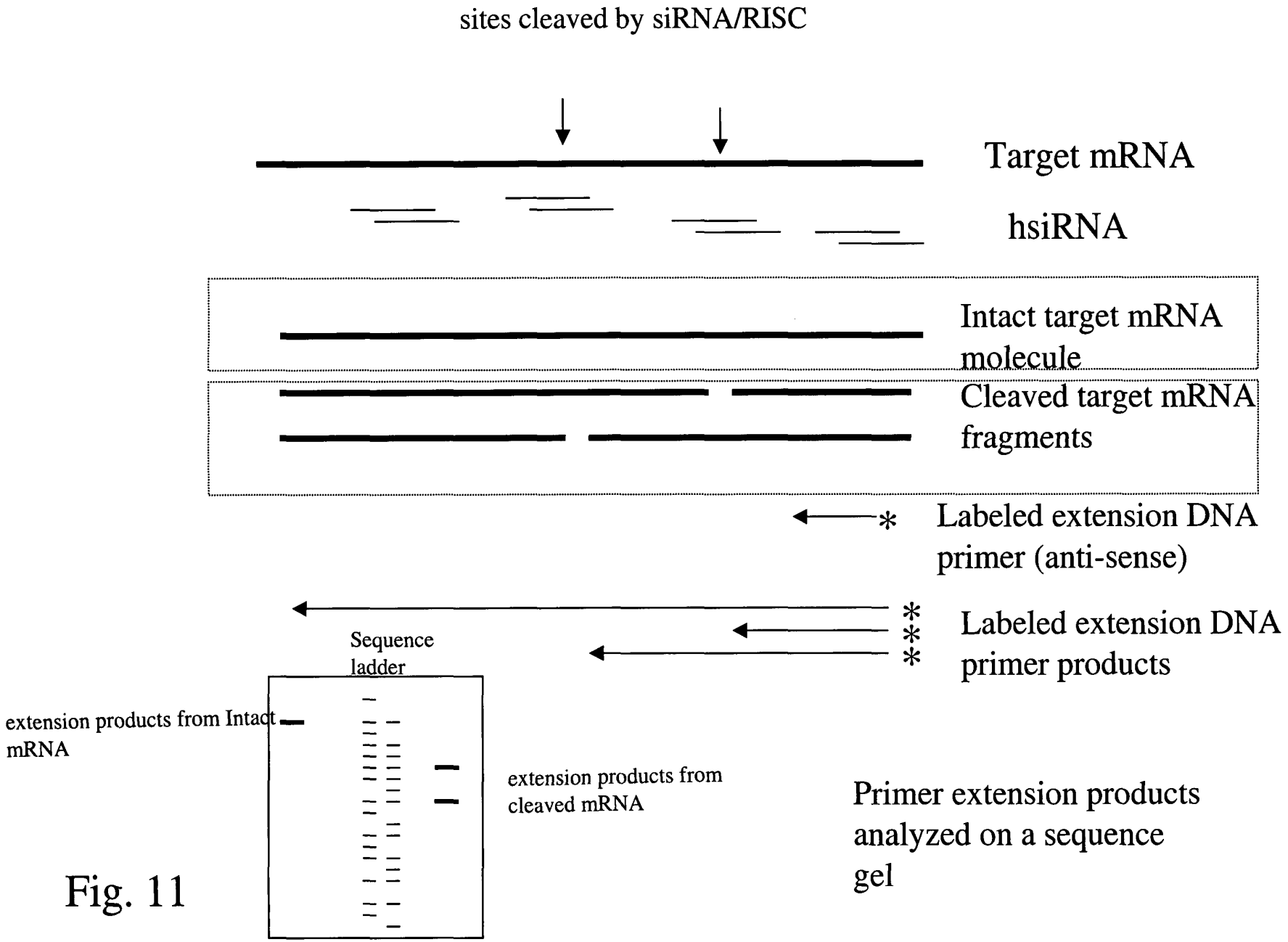

sites cleaved by siRNA/RISC
Target mRNA
hsiRNA
Intact target mRNA molecule
Cleaved target mRNA fragments
Labeled extension DNA primer (anti-sense)
Labeled extension DNA primer products
Sequence ladder
extension products from Intact mRNA
extension products from cleaved mRNA
Primer extension products analyzed on a sequence gel

Fig. 11